STUDIES ON THE RELATIVE SYNTHESIS AND DISTRIBUTION OF IgA AND IgG1 IN VARIOUS TISSUES AND BODY FLUIDS OF THE COW

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In vitro synthesis of bovine IgA was detected by a radioimmunoelectrophoretic assay in 17 of 20 different tissues obtained from cows and 2 of 6 different tissues obtained from a 1-week-old calf. Conclusions drawn from these qualitative studies are supported by the results of preliminary quantitative data. Synthesis of IgA was most pronounced in the adult ileum, duodenum, colon, lungs, lower nasal mucosa, oral pharyngeal mucosa, parotid gland, lacrimal gland and thymus and the calf parotid and lacrimal glands. Correspondingly, IgA, not IgG1, is the principal immunoglobulin in bovine tears and saliva. In comparison to other secretory tissues of the cow and the mammary glands of the rabbit, human and monkey, the bovine mammary gland shows moderate deficiency in the synthesis of IgA. This deficiency is reflected in the fact that IgA does not become the predominant immunoglobulin of normal bovine milk, although an increase in the IgA to IgG1 ratio does occur after parturition. The adult bovine thymus is an immunologically active secretory tissue, but the ileum of the 1-week-old calf appears to lack an IgA-producing system. The concentration of serum IgA is lower than in the human and is greater before calving than after. Most of the IgG1 synthesized in the mammary gland is eluted from Sephadex G-200 before 7S IgG1.

The principal immunoglobulin in the exocrine secretions of man and of most other mammals is secretory IgA (SIgA) (1). By contrast, IgG1 is the principal immunoglobulin of bovine milk and colostrum (2) and has been reported by some to predominate in saliva (3). Bovine SIgA has been identified recently (4–7). Because the presence of an immunoglobulin in a secretion is not direct evidence of its synthesis by the organ sequestering the secretion, *in vitro* studies of immunoglobulin synthesis were also undertaken. This paper presents the results of qualitative and preliminary quantitative studies of bovine immunoglobulin synthesis. In addition, clarification of the quantitative relationship of bovine SIgA and IgG1 is presented for five body fluids sampled before and after parturition.

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MATERIALS AND METHODS

Collection of body fluids. Weekly samples of serum, saliva, tears, urine and lacteal secretions from individual mammary gland quarters were taken from six Holstein-Friesian cows at the same time each week for 10 weeks encompassing the time before and after parturition. The casein was removed by acid precipitation from all lacteal samples. Radial diffusion analyses of reconstituted precipitates and supernatants indicated that 5 to 7% of each

immunoglobulin class was precipitated with the caseins. Samples of all fluids were taken directly without chemical stimulation. Total protein was determined by the biuret method (8).

Preparation of immunoglobulins. SIgA, IgG1 and IgG2 were prepared as described elsewhere (9–11). Each preparation formed a single but distinct immunoelectrophoretic arc when tested with a polyvalent antiserum (9) (see below). IgG1 and IgG2 produced single zones after polyacrylamide electrophoresis at pH 4.5 (9) while SIgA showed a distinct major zone and also a faint zone corresponding in position to IgG1 (9). Single radial diffusion analyses using the monospecific antisera described below indicated that IgG1 and IgG2 contained no detectable contaminating proteins but that SIgA contained 20% IgG1. The amount of IgG1 was subtracted from the total protein concentration of the SIgA preparation when standard curves were established in radial diffusion assays of this protein.

Preparation of antisera. Antisera to SIgA, IgM, α-2 macroglobulin, lactoferrin, β-lactoglobulin and to glycoprotein-a (Gpa), the free secretory component (FSC) of SIgA (5, 10, 12, 13), were prepared as described elsewhere (9). The antiserum to SIgA did not react with Gpa in radial diffusion. The antiserum to Gpa gave a reaction of partial identity with SIgA (9, 10). Antisera to IgG, IgG1 and bovine serum albumin (BSA) were prepared by immunizing rabbits with Fab, IgG1 and BSA, respectively, in Freund's complete adjuvant. Antiserum to IgG1 was rendered specific by absorption with IgG2 insolubilized with glutaraldehyde (14). Three different polyvalent antisera were prepared in rabbits and used in this study. Anti-immunoglobulin sera (P and P1) were prepared by immunization with that fraction of colostral whey insoluble in 50% ammonium sulfate. Such antisera also contained antibodies to α-2 macroglobulin, certain lipoproteins, macromolecular component (1) and occasionally Gpa. Antiserum prepared against the fraction of whey soluble in 50% ammonium sulfate (M) precipitated the major non-immunoglobulin protein of whey, *i.e.*, β-lactoglobulin, α-lactalbumin, bovine serum albumin and lactoferrin. The properties of these antisera are further described where used.

Immunochemical analyses. Immunoelectrophoresis and immunodiffusion were performed as described elsewhere (9, 10). The concentration of total IgG, IgG1, BSA and IgA were determined by the method of Fahey and McKelvey (15). The standard curve for IgA was obtained by using an 11S SIgA preparation from colostrum in which the total protein concentration was determined by absorbance [$E^{1\%}_{280nm}$, 1 cm = 13.7] and the amount of contaminating IgG1 by radial diffusion. The extinction value used is that determined for bovine IgG and compares favorably with reported values for both human IgG and IgA (16). The various body fluids were concentrated or diluted as necessary and 2 to 4 determinations were made on each sample.

In vitro synthetic studies. Tissues were obtained at slaughter from two lactating cows and a 1-week-old calf. Minced tissue was incubated for 18 hr in roller tubes in a medium (17) containing ^{14}C-labeled lysine and isoleucine (lots B35, 312 mCi/mM and B20 312 mCi/mM, Amersham/Searle Corp., Des Plaines, Ill.). Culture fluids were centrifuged, dialyzed against 0.015 M NaCl, lyophilized and reconstituted to one-tenth of their original volume with distilled water. Portions of concentrated culture fluids were added either to a mixture of serum and colostral IgA or to whey and subjected to immunoelectrophoresis. The unlabeled mixtures of proteins served as "carriers" to ensure sharp precipitin arcs. Precipitin patterns were developed with appropriate antisera. Autoradiographs were prepared of the washed, dried immunoelectrophoretic patterns as described by Hochwald, Thorbecke and Asofsky (17). Labeled specific proteins were identified by comparing the autoradiographs with the stained patterns. The degree of labeling was estimated visually, on a scale of 0 to 4+, from the intensity of the autoradiographic image.

Preliminary quantitative synthetic studies. Tissues were cultured as described previously except that Erlenmeyer flasks were used in place of roller tubes. The reconstituted supernatants were mixed with a suitable amount of colostral whey or tears and the mixture dialyzed against a 0.1 M Tris, pH 8.3, buffer containing 0.9 M NaCl, 0.02% sodium azide and 0.003 M EDTA and applied to a 2.5- x 90-cm

upward flowing column of Superfine Sephadex G-200. Protein in the eluant was monitored by absorption at 282 nm (LKB Uvicord II) and the distribution of various proteins determined by single radial diffusion against appropriate antisera. The distribution of total radioactivity was determined by adding 0.5 ml of each 6-ml fraction collected to 9.0 ml of a toluene-Triton X-100 scintillation cocktail. Each sample was counted for 10 min in a Packard Tri-Carb automatic counter. To obtain samples large enough for repeated study, we pooled the fractions containing SIgA and IgM (A1), 7S IgG (B) and Gpa and lactoferrin (C), respectively (Table III). Fractions intermediate between the elution of SIgA and 7S IgG (A2, A3) were pooled from mammary culture fractions because pilot studies suggested significant amounts of labeled IgG in this region. The optimal antigen-antibody ratio for each pool was determined for rabbit anti-IgG1, Gpa, IgM, IgA and lactoferrin. The amount of protein-bound radioactivity was determined by precipitation with trichloroacetic acid (TCA). Specific radioactivity was precipitated in slight antibody excess with the above antisera. Duplicate or triplicate precipitin tests were incubated 1 hr at 37°C and 3 days at 4°C. The precipitates were washed three times with cold saline, dissolved in 0.1 M NaOH and combined with 9 ml of the scintillation cocktail. Complete reciprocal precipitation of the supernatants was limited by the supply of antisera.

RESULTS

The levels of IgA, IgG1, IgG, BSA and total protein found in prepartum and postpartum secretions are shown in Table I. As indicated by the standard errors, variations in levels of these parameters among the prepartum specimens and among the postpartum specimens were rather small. IgA, not IgG1, was the principal immunoglobulin in two of the three external secretions tested, saliva and tears, and a minor constituent of the lacteal secretions. IgA accounted for a high percentage of the total protein in tears and saliva but not in milk or serum. The highest mean level of IgA was found in tears; a few specimens had levels as high as 8.0 mg/ml. Concentration of IgA in precolostral samples from some quarters reached levels as high as 9.0 mg/ml. One an-

TABLE I

IgG, IgG1, IgA, BSA and total protein levels in the body fluids of cows

Body Fluid	No. Samples	Concentration, mg/ml Mean ± S.E.M.					IgA/IgG1 Ratio	IgA % of Protein
		IgG[a]	IgG1	IgA	BSA	Protein		
Prepartum								
Serum	36	20.3 ± 0.8	10.7 ± 1.2	0.10 ± 0.008	32.02 ± 0.05	61.2 ± 1.6	0.005[b]	0.16
Lacteal secretions[c]	144	37.4 ± 1.6	33.8 ± 1.7	1.83 ± 0.14	1.45 ± 0.4	50.9 ± 1.4	0.054	3.59[d]
Saliva	36		0.05 ± 0.01	0.30 ± 0.04	013 ± 0.0	1.44 ± 0.43	6.0	21.0
Tears	36		0.54 ± 0.08	3.88 ± 0.49	0.58 ± 0.32	8.96 ± 0.79	7.2	43.0
Urine	18		0.017	0.0019			0.11	
Postpartum								
Serum	24	26.4 ± 1.7	12.9 ± 1.4	0.06 ± 0.01	28.34 ± 0.78	61.5 ± 1.3	0.002[b]	0.10
Lacteal secretions[c]	96		1.2 ± 0.57	0.23 ± 0.05	0.45 ± 0.36	12.3 ± 0.69	0.19	1.87[d]
Saliva	24		0.03 ± 0.01	0.17 ± 0.05	0.006 ± 0.004	1.3 ± 0.47	5.6	13.0
Tears	24		0.48 ± 0.07	3.75 ± 0.58	0.48 ± 0.27	8.24 ± 1.11	7.8	45.0

[a] The relatively large amounts of Fab and $F(ab)_2$ fragments in tears, saliva and normal milk (9) compared to 7S IgG did not permit accurate quantitation of total IgG in these secretions.

[b] Ratio of IgA/total IgG.

[c] Absolute values are not corrected for random loss of immunoglobulins resulting from their coprecipitation with casein.

[d] Percentage of total whey protein.

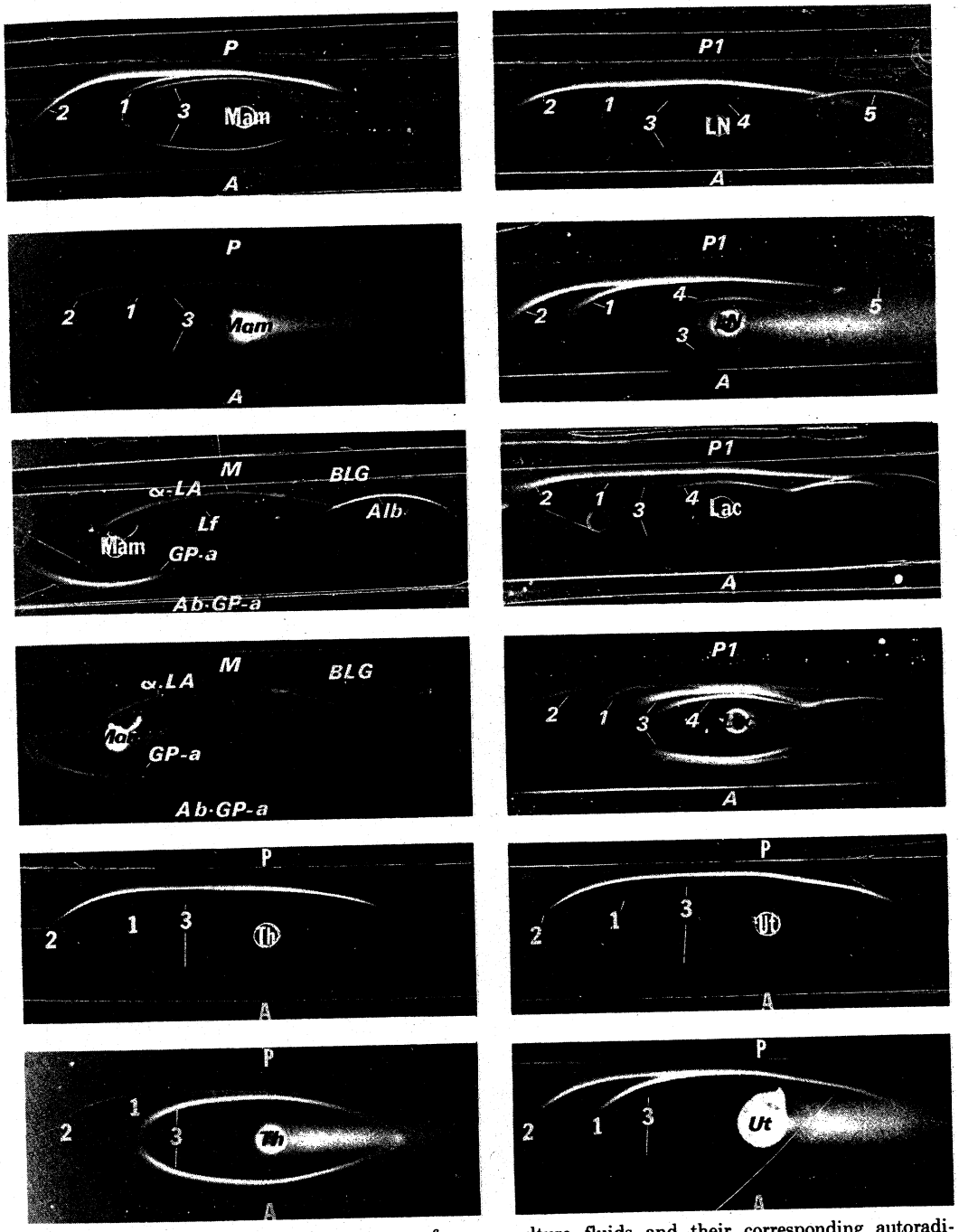

Figure 1. Immunoelectrophoretic patterns of organ culture fluids and their corresponding autoradiographic images. Anode is at the left. *LN*, lymph node; *Th*, thymus; *Lac*, lacrimal gland; *Ut*, uterus; *Mam*, mammary gland parenchyma. (White letters designate the stained slides whereas black letters designate the autoradiographs.) *P*, Polyvalent antiserum for IgG1, IgG2 and IgA; *A*, antiserum specific for IgA; *Pl*, polyvalent antiserum for IgG1, IgG2, IgA, IgM and α-2 macroglobulin; *M*, polyvalent antiserum to 50% ammonium sulfate soluble whey proteins; *Ab-Gpa*, antiserum to Gpa; *Gp-a*, Glycoprotein-a; *α-LA*, α-lactalbumin; *Lf*, lactoferrin; *BLG*, β-lactoglobulin; *Alb*, serum albumin; *1*, IgG1; *2*, IgG2; *3*, IgA; *4*, IgM, and *5*, α-2 macroglobulin.

imal had a mean prepartum IgA concentration of 5.0 mg/ml. Blood IgA levels never exceeded 0.3 mg/ml and remained relatively constant.

IgA to IgG1 ratios, rather than absolute levels, have been routinely used in human beings (1) as an index for comparison of the distribution of immunoglobulins in body fluids. This circumvents problems arising from the variable dilution factors present in most secretions, since all proteins presumably will be diluted to the same extent. The IgA to IgG1 ratio was high in tears and saliva but very low in serum and lacteal secretion. There was a marked change in the ratio of IgA to IgG1 in lacteal secretions before and after calving and a small inverse change in serum (Table I). Albumin levels were only 3-fold lower after calving while IgG1 levels were nearly 30-fold lower.

Representative results of qualitative *in vitro* synthetic studies are illustrated in Figure 1; data from all cultures are summarized in Table II.

The distribution of specific proteins and radioactivity after chromatography of lacrimal gland culture-tears and mammary gland parenchyma-colostral whey mixtures on Sephadex G-200 are shown in Figure 2. Except for the radioactivity eluted just before the first protein peak (IgM–IgA peak), most of the radioactivity was protein-bound (Table III). Despite the variability among mammary parenchyma cultures, most radioactivity was bound to proteins eluted with the first protein peak. This was particularly true in the lacrimal gland culture. Table III presents the results of preliminary coprecipitation studies with various antisera. The data are considered preliminary because the availability of suitable monospecific antisera limited complete reciprocal precipitation of supernatants as well as restricted the number of determinations to less than that needed for statistical evaluation. Similarly, although the distribution of radioactivity for three different mammary cultures is shown in Figure 2, the coprecipitation data in Table III were obtained from only one of these cultures. Between 70 and 100% of the total reactivity was recoverable in either TCA supernatants or precipitates, but the total TCA precipitable radioactivity was not recoverable by coprecipitation with the antisera employed. In both tissues about one-half of the TCA-precipitable radioactivity in the first peak was coprecipitated with either anti-IgA (α-chains) or anti-Gpa. The descending shoulder of the first peak (pools A2 and A3) from mammary cultures showed a surprisingly large amount of radioactivity precipitable with anti-γ1-chains.

TABLE II

Qualitative evaluation of in vitro synthetic studies

Tissue	Relative Intensity[a] of Radioimmunoelectrophoretic Arcs			
	IgA	IgG1	IgG2	IgM
Mammary gland parenchyma[b, c]	0.6	0.6	1.0	0.4
Mammary gland cistern[c, d]	0.3	1.0	1.3	1.0
Thymus	3,4	1,1	2,1	1,1
Lacrimal gland	4,3	1,1	0,1	1,1
Upper nasal mucosa	2	2	2	1
Lower nasal mucosa	3	1	1	1
Oral pharynx	2	1	1	1
Ileum	3,3	1,1	1,1	1,2
Duodenum	3,3	1,1	1,1	1,1
Colon	3	1	1	2
Parotid gland	2,2	1,0	0,1	1,1
Lung	2,3	2,1	1,2	0,1
Adrenal cortex	0	1	0	0
Kidney cortex	0	1	0	0
Uterus	1,2	3,2	3,1	0,0
Vagina	2	2	1	1
Femoral lymph node	0,0	3,2	3,3	1,1
Supramammary lymph node	0,1	2,3	2,3	1,1
Mesenteric lymph node	1,1	2,1	1,1	1,1
Spleen	1,1	2,1	3,2	2,3
Average of all lymph nodes and spleen	0.6	2.1	2.5	1.2
Calf spleen	0	2	1	1
Calf parotid gland	3	1	0	1
Calf lacrimal gland	2	1	0	0,1
Calf thymus	0	0	0	0
Calf ileum	0	0	0	0
Calf lung	0	0	0	0

[a] The intensity of the autoradiographic images were graded visually on a scale of 0 (invisible), 1 (just visible) to 4 (very dark).

[b] Average of five determinations on tissues from two cows. All parenchyma samples showed intense labeling of β-lactoglobulin and α-lactalbumin.

[c] Average values <1.0 result from negative results being assigned a numerical value of 0 so that, *e.g.*, a 1 and a negative equal 0.5.

[d] Average of three determinations on tissues from two cows. No labeling of β-lactoglobulin and α-lactalbumin was observed.

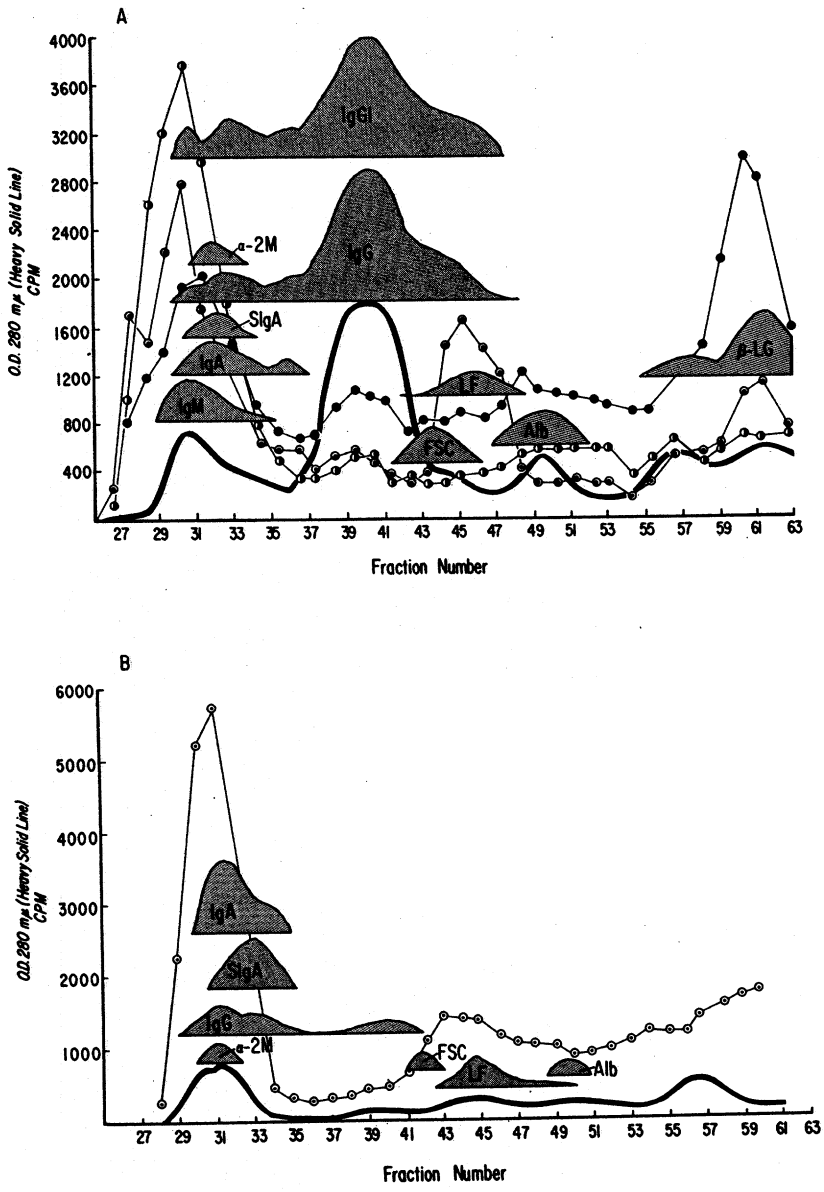

Figure 2. Distribution of proteins and radioactivity in supernatants from bovine mammary gland parenchyma (*A*), and lacrimal gland (*B*) cultured *in vitro* with ^{14}C-labeled amino acids. Heavy line = absorbancy at 282 nm of eluted protein. ⊙, ◑, ● = Distribution of radioactivity from three different mammary gland cultures or one (⊙) lacrimal gland culture. Distribution of specific proteins determined by radial diffusion. *FSC*, free secretory component (Gpa); *Lf*, lactoferrin; *α-2M*, α-2 macroglobulin; *β-LG*, β-lactoglobulin. Quantitative coprecipitation data in Table III obtained from the culture designated by ⊙.

Because pilot studies showed that little or no radioactivity could be coprecipitated with anti-γ1-chains in equivalent fractions from the lacrimal gland, data from parallel pools are not included in Table III. The majority of the radioactivity in pool C from lacrimal gland was accountable as lactoferrin but most of the radioactivity in the equivalent fraction from mammary gland cultures was not coprecipitable with any of the antisera used.

TABLE III

Radioactivity (CPM) precipitable with TCA and specific antisera in pooled fractions eluted from G-200

	Mammary Gland Parenchyma				
	Pool A1	Pool A2	Pool A3	Pool B	Pool C
Total	2280	2005	755	520	600
TCA pptd.	1683	1620	400	470	580
TCA soluble	358[a]	398	270	47	0
Anti-Gpa	903[b]	521	155	31	56
Anti-IgA (α-)	1050	428	94	108	10
Anti-IgG1 (γ1-)	181	426	137	123	86
Anti-IgM (μ-)[a]	346	ND[c]	120	51	20
Anti-Lf	ND	ND	ND	0	72

	Lacrimal Gland		
	Pool A	Pool B	Pool C
Total	3145	420	1372
TCA pptd.	2600	250	950
TCA soluble	218	40	154
Anti-Gpa	1160	0	72
Anti-Iga (α-)	1062	ND	ND
Anti-IgG1 (γ1-)	125	0	ND
Anti-IgM (μ-)	42	0	ND
Anti-Lf	ND	0	650

[a] Some α-2 macroglobulin and IgA radioactivity have coprecipitated with this antisera.

[b] Overbar indicates that value is mean of more than duplicate determinations.

[c] ND, not done.

DISCUSSION

The quantitative relationship between IgG1 and IgA is consistent with that recently reported by others (13, 18). The serum IgA levels were lower than those reported in Simmental (13) and Ayrshire (6) cattle but similar to those reported by others in Holstein (Wilson, M. R., Duncan, J. R., Heistand, F. and Brown, P., Personal communications, 1971). The inverse changes in the levels of IgG1 in serum and milk before and after calving are similar to those first described by Blakesmore and Garner (19) and suggest a slow down in the rate of selective IgG1 transport (20) from serum to milk after calving. This hypothesis is also supported by the finding that 66% of the total whey protein before calving is IgG1, but only 10% of normal milk whey is IgG1 (Table I). The selective transport of IgG1 is confirmed by the observation that it comprises nearly all of the total IgG in colostrum but only half of that in serum, and by the small drop in BSA concentrations after calving compared to the large drop in IgG1 concentration. The change in the ratio of IgA to IgG1 in lacteal secretions before and after calving, combined with the low level of IgA in serum, is consistent with the view that IgA is locally synthesized in the udder (Table I). The corresponding inverse change in the IgA to IgG1 ratios in serum reflects the higher IgG1 and lower IgA levels after calving.

Evaluation of the qualitative data in Table II reveals several patterns of immunoglobulin synthesis among the different tissues. For example, when two tissues show the same labeling intensity for IgG1 (mammary gland cistern and thymus) but greatly different intensities of IgA labeling (0.3 *vs* 3, 4), it is valid to conclude that the thymus synthesizes much more IgA than the mammary gland cistern relative to the same amount of IgG1 synthesis and that mammary gland cistern and thymus demonstrate two different patterns of immunoglobulin synthesis. The validity of the last method of comparison is supported by the preliminary quantitative data presented in Figure 2 and Table III and made possible the distinguishing of four general patterns of synthesis (Table II, Fig. 1): 1) tissues in which IgA precipitin arcs were intensely labeled; 2) tissues in which IgG1, IgG2 and IgM precipitin arcs were strongly labeled; 3) tissues in which all immunoglobulin precipitin arcs were weakly to moderately labeled; and 4) tissues in which labeling of precipitin arcs was faint or absent.

The first pattern is similar to synthetic and histologic patterns described for human, monkey and rabbit tissues that comprise the secretory immune system and which are known to synthesize predominantly IgA (1, 21–25). In this study, these tissues fit such a pattern: ileum, duodenum, colon, lungs, lower nasal mucosa, oral pharyngeal mucosa, parotid and lacrimal glands of the calf. Eight samples from various regions of the mammary gland of two cows were analyzed. No striking regional variations were observed among samples taken in the parenchyma, but labeling of IgG1 and IgG2 were more pronounced in samples from the cistern area than those taken in the parenchyma area. In all samples, the intensity of the labeled immunoglobulin arcs, especially IgA,

was lower than in most other tissues and hence the pattern of synthesis differed from that of the human, rabbit and monkey (24) and from secretory tissues located elsewhere in the cow. The preliminary quantitative synthetic data in Figure 2 and Table III indicate that while more IgA than IgG1 is synthesized by the mammary gland the ratio of IgA to IgG1 synthesis is much greater in the lacrimal gland ($\simeq$8 to 1) than in the mammary gland ($\simeq$2:1). The distribution of the labeled IgG1 in mammary gland cultures suggests it may be a dimer (Fig. 2). Immunofluorescent studies of the same mammary tissue showed considerable IgG-containing cells (26).

The quantitative predominance of IgG1, especially in bovine colostrum (Table I), is understandable in view of the type of maternal-fetal transfer of immunoglobulins characteristic of the cow compared with the type used by human, monkey and rabbit (2, 27, 28) but the continued predominance of IgG1 in normal milk is more difficult to explain. In horses and pigs (7), which also transfer immunity via IgG in their colostrum, IgA is also a minor component in colostrum, but becomes the principal immunoglobulin in normal milk after neonatal absorption of immunoglobulins has ceased. In all ungulates, intestinal absorption by the neonate ceases after about 48 hr (28). Similar, but less spectacular changes occur in species like rodents, cats and dogs that transfer IgG both *in utero* and via colostrum but IgA always predominates in milk after IgG transfer ceases (7, 27). The IgA-IgG1 relationship in normal milk, together with the qualitative and quantitative synthetic data support the concept of an apparent IgA-deficient system in the bovine mammary gland. The failure of the bovine mammary gland to display a pattern of immunoglobulin synthesis which resembles that of tissues comprising the secretory immune system may be explained by recent studies of human beings with IgA deficiencies. Such persons absorb macromolecules across the intestinal mucosa to a much greater degree than normal people (29–31). The role of IgA in maintaining membrane integrity has also been suggested from studies of respiratory viruses (32). It may be that the existence of a well developed IgA-mediated secretory immune system would be incompatible with the physiology of an organ secreting large amounts of protein across membranes over an extended period. Such is the case of the lactating mammary gland of the domestic cow. Recent studies on the sheep, a related ruminant, support this contention (33). The involuted mammary glands of ewes, which are not actively transporting protein, contain more IgA plasma cells and more readily synthesize IgA than the lactating gland.

The synthesis of IgA by the adult thymus is consistent with recent studies showing fluorescence in Hassall's corpuscles of the cow and man by using fluorescein-conjugated antisera to bovine and human IgA (26).

The second pattern of immunoglobulin synthesis observed is characteristic of the central lymphoid tissue of all species studied (1, 21–25). The adult spleen and lymph nodes and calf spleen revealed such a pattern. Pattern 3 was observed in the upper nasal mucosa, uterus, vagina and the mammary gland. The fourth pattern observed is characteristic of tissues that do not typically synthesize immunoglobulins, those obtained from agammaglobulinemic persons or those obtained from immunologically immature animals (34–36). The calf ileum, thymus and lungs, and the adult adrenal and kidney fit into such a pattern.

It was difficult to distinguish newly synthesized Gpa from newly synthesized IgA by the techniques used. In the mammary gland, the characteristically shorter precipitin arc of Gpa developed with anti-Gpa (Fig. 1) often appeared outside of the SIgA arc (9, 10, 13), differed from the precipitin arc of IgA that was developed with anti-IgA (anti-IgA did not precipitate with Gpa) and was labeled in mammary cultures. The fact that much of the newly synthesized Gpa may be complexed with carrier or newly synthesized IgA leaves the issue of their quantitative synthetic relationship unresolved. Labeled FSC was demonstrated in lacrimal and mammary gland cultures by gel filtration and coprecipitation (Fig. 2, Table III).

Gpa was first isolated from normal milk (37) and is known to be particularly abundant in this secretion (9, 13, 38). Because the presence of excess secretory component is characteristic of humans with IgA deficiencies (1) its abundance in cows' milk may be related to the IgA-hypogammaglobulinemia demonstrated by this organ.

IgA synthesis in the human (36) and cattle (39) does not develop before birth and is first apparent in piglets after 1 week of age (40). The apparent absence of IgA synthesis in the calf gut compared to the lacrimal gland may be due to the absence of antigenic stimulation and may allow for proper absorption of maternal immunoglobulins to occur.

The finding of two labeled precipitin lines for IgA in lacrimal gland cultures (Fig. 1) is consistent with the physicochemical and antigenic heterogeneity recently described for lacrimal IgA (10). The ubiquitous synthesis of IgG by various bovine tissues (Table II) and the evidence for the dimeric nature of the IgG in mammary cultures suggest that IgG may be more important in cattle than in man and that its contribution to the secretory immune system should not be ignored.

Acknowledgments. We thank Barbara Ison, Toni Beach, Joseph Schwartz and Charles Caudill for statistical analyses of the data and Lawrence Adler for technical assistance.

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